

Figure 1a

MDSEAFQSARDFLDMNFQSLAMKHM DLKQMELDTAAKVDELTQLESWSDSPAPP GPQAGP
 PSRPPRYS SSSSIPEPFGSRGSPRKAATDGADTPFGRSESAPLH PYSPLS PKGRSSPR TPLYLQPDAY
 GS LDRATSPR PRAFDGAGSSLGRAPS PRPGPGLRQQGPPTPDFLGRAGS PRGSPLAEGPQ AFFPE
 RGPS PRPPATAYDAPASAFGSSLLGGSGSAFAPPLRAQDDLT LRRR PPKAWN ESDLDV AYEKKPSQ
 TASYERLDVFAR PASPLQLLPWRESSLDGLGGTGDNLTSATLPRNYKV SPLAS DR RSDAGSY RR
 SLGSAGPSGTLPRSWQPVS RIPMPPSSPQ PRGAPQR PPIPLSMIFKLQNAFWEHGASRAMLPGSPLF
 TRAPPK LQ P QP QP QSQ P QP QP QT QP QT PPAQHPQQT WPPVNEGPPK PPT EPEPEI
 EGLLTPVLEAGDVDEGPVARPLSPTRLOPALPPEAQSVPEEVARV LAEIPRPLKRRGSMEQAPA
VALPPTHKKOYQQISRLFHRHGGPGGGPEPELSPITEGESEARAGPPAPAPPAPIPPPAPSOSSPPEQ
POSMEMRSVLRKAGSPRKARRARLNPLVLLDAALTGELEVVOQA VKE MNDPSOPNEEGITALH
NAICGANYSIVDFLITAGANVNSPDSHGWTPLHCAASCNDTVICMALVQHGAIAFATT LSDGATAF
EKCDPYREGYADCATYLADVEQSMGLMNSGAVYALWDYSAEFGDELSFREGESVTLRRDGPEE
TDWWWAALHGQEGYVPRNYFGLFPRVKPORSKV*

Figure 1b

CCACCGGTCCGGGAAGCCCCCAGGTGCCAGGATCTGCCCGATCCGCCCGCTCCGCCGG
 CACCATGGACAGCGAGGCATTCCAGAGCGCGCGGACTTCTGGACATGAACCTCCAGTCGCT
 GGCCATGAAACACATGGATCTGAAGCAGATGGAGCTGGACACGGCGGCCAAGGTGGATG
 AACTGACCAAGCAGCTGGAGTCGCTGTGGTACAGACTCTCCAGCTCGATCCCTGAGCCCTTCGGCAGCCGAG
 GACCCCTCTAGGCCGCCCCGGTACAGCTCCAGCTCGATCCCTGAGCCCTTCGGCAGCCGAG
 GGTCCCCCGGAAGGCGCCACCGACGGCGAGACACCCCGTTCTGGACGGATCAGAGAGTGCC
 CCAAC CCTACACCCCTACAGCCC GTGTCCCCAAGGGACGGCGCTCGCCGCCACCCCG
 CTCTACCTGCAGCGGACGCCCTACGGCAGCCTGGACCTCGCCCTCCCGGGCCAGGCCGCTC
 TTCGATGGCGCAGG CAGCTCCCTGGCCGTGCGCCCTCCCGGGCCAGGCCGCTC
 CGCCAGCAGGGTCCCCCA CGCCTTTCGACTCCTGGCCCGCAGGCTCCCCCGCCAGC
 CCCCTGGCGGAGGGGCCCCAGGCCTTCTCCCCAGCGTGGCCGTACCCGCCCCCTGCC
 ACAGCTACGACCGCCAGCGTCCGCCCTGGGAGCTCCCTGCTAGGCTCCGGCGCAGCGCA
 TTCGCCCGCCTCTGC CGCGCAAGACGACCTGACGCTGCGCCGGCCTCCGAAAGCCTGG
 AACGAGTCTGACCTGGACGTGGCTACGAGAAGAAGCCTTCGAGACAGCGAGCTATGAACG
 CCTGGACGTCTCGCAAGGCCTGCCCTCGCCAGCCTGAGCTGCTGCTTGGAGGGAGAGCAG
 CCTGGATGGACTGGGGGCACCGGCAAGGACAACCTCACTAGCGCCACCCCTGCCGCGCAATT
 ACAAGGTCTCTCTGGCCAGCGACCCGGCTTCAGACCGCGGAGCTACC GGCGCTCGCTGG
 GCTCCGCGGGCCGTGGCACTTGCCTCGCAGCTGGCAGCCCGTACCCGCACTCCCATGC
 CCCCCCTCCAGCCCCCAGCCCCCGGGGCCCGCCAGCGTCCCATCCCCCTCAGCATGATCT
 TCAAGCTGCAGAACGCCCTCTGGGAGCACGGGCCAGCCGCCTGCTCCCTGGTCCCCC
 TCTTCACCGAGCACCCCGCTAACGCTGCAGCCCCAACACAACCAGCCCCAGCCACAAT
 CACAACACAGCCCCAGCTGCCCAACAGCCCCAGACCCAACCCAAACCCCTACCCAGCCC
 CCCAGCATCCCCAACAGACATGGCCCCCTGTGAACGAAGGACCCCCAAACCCCCACCGAG
 CTGGAGCCTGAGCGGAGATAGAGGGGCTGCTGACACCAGTGTGGAGGCTGGCGATGTGGA
 TGAAGGCCCTGTAGCAAGGCCTCTAGCCCCACGAGGGCTGCAAGCCAGCAGCAGTGCACCGGAGG
 CACAGTGGTCCCCAGCTGGAGGGATCTGAGGGCAGGGCAGGGCCCCCTGCTCTGCC
 AAACCGAGGGCTCCATGGAGCAGGCCCTGCTGTGGCCCTGCCCTACCCACAAGAAACA
 GTACCAGCAGATCATCAGCCGCTTCCATCGTCACTGGGGCCAGGGCCGGGGCCGG
 AGCCAGAGCTGCCCCCATCACTGAGGGATCTGAGGGCAGGGCAGGGCCCCCTGCTCTGCC
 CACCAAGCTCCCATTCCACCCCGGGCCCGTCCAGAGCAGCCCACAGAGCAGCCAGAGC
 ATGGAGATGCGCTCTGTGCTGCCAGAGCGGGCTCCCGCGCAAGGCCCGCCGCGCGCCT
 CAACCCCTCTGGTGTCTCCCTGGACCGCGCGCTGACCGGGGAGCTGGAGGTGGTGCAGCAGG
 CGGTGAAGGAGATGAACGACCCGAGCCAGCCAAACGAGGAGGGCATCACTGCCTTGACAAAC
 GCCATCTGCGGCCAACACTACTCTATCGTGGATTCCCTCATCACCGCGGGTGCACATGCAAC
 TCCCCCGACAGCCACGGCTGGACACCCCTGCACTGCGCGCGTGTGCAACGACACAGTCATC
 TGCA TGGCGCTGGTGCAGCACGGCGCTGCAATCTCGCCACCACGCTCAGCGACGGCGCACC
 GCCTCGAAGTGCACCCCTACCGCGAGGGTTATGCTGACTGCGCACCTACCTGGCAGAC
 GTCGAGCAGAGTATGGGGCTGATGAACAGCGGGCAGTGTACGCTCTGGACTACAGCGC

CGAGTTGGGGACGAGCTGTCCCGAGGGCGAGTCGGTCACCGTGCAGGGAGGGACGG
GGCCGGAGGAGACCGACTGGTGGTGGGCCGCTGCACGGCCAGGAGGGCTACGTGCCGCC
AACTACTCGGGCTGTTCCCAGGGTGAAGCCTCAAAGGAGTAAAGTCTAGCAGGATAGAAG
GAGGTTCTGAGGCTGACAGAAACAAGCATTCTGCCTCCCTCCAGACCTCTCCCTCTGTTT
TTGCTGCCCTTATCTGCACCCCTCACCCCTGCTGGTGGTCCCTGCCACCGTTCTGTCTC
CTGGAAGTCCAGGGAGAAGGAGGGCCCCAGCCTAAATTAGTAATCTGCCCTAGCCTGGG
AGGTCTGGGAAGGGCTGAAATCACTGGGACAGGAAACCACCTCCTTTGCCAAATCAGAT
CCCGTCCAAAGTGCCTCCCATGCCTACCACCATCATCACATCCCCAGCAAGCCAGCCACCTG
CCCAGCCGGGCTGGATGGGCCACCAACCCACTGGATATTCTGGGAGTCACTGTCACACC
ATCTCTCCCAGCAGTCTGGGTCTGGTGGAAACATTGGTCTTACCAAGGATCCCTGCC
ACCTCTCCCCAATTAAGTGCCTCACACAGCTCTGGTTAATGTTATAAACAAAATAGAGAA
ACTTTCTTATAAATAAAGTAGTTGCACAGAAAAAAAAAAAAAA

Figure 2a

MWMKDPVARPLSPTRLQPALPPEAQSVPELEEVARVLAIEPRPLKRRGSMEQAPAVA
 LPPTHKKQYQQIISRLFHRHGGPGGGRSQSCPSSLRDLRPGQGPLLPHQLPFHRPAP
 SQSSPPEQPQSMEMRSVLRKAGSPRKARRARLNPLVLLDAALTGELEVQQAVKE
 MNDSQPNEEGITALHNAICGANYSIVDFLITAGANVNSPDSHGWTPHLHCAASCNDT
 VICMALVQHGAAIFATTLSDGATAFEKCDPYREGYADCATYLADVEQSMGLMNSGA
 VYALWDYSAEFGDELSFREGESVTLRRDGPEETDWWWAALHGQEYVPRNYFGL
 FPRVKPQRSKV

Figure 2b

GCAGGGCCGCGTCGACCCGGCGTTCAGACGCCAGCTACCGCGCTCGCTGGGTCCGCCGGCG
 GGGCACTTGTCTCGCAGCTGGCAGCCGTCAGCCGATCCCAGCCCCCTCCAGCCCCAGCCCC
 GCAGGGCCCGCGCCAGCGTCCCACATCCCCCTCAGCATGATCTCAAGCTGCAGAACGCCCTCTGGGA
 GCACGGGCCAGCCGCG CCATGCTCCCTGGGCCCCCTTCACCCAGCAGCCCCAGCTGCCAACAGCCCC
 CAGCCCCAACACAAACCACAGCCCCAGCCACAATACAACACAGCCCCAGCTGCCAACAGCCCC
 AGACCCAACCCAAACCCCTACCCCAGCCTCCCACATCCGATCCCCAACAGACATGGCCCCCTGTG
 AACGAAGGACCCCCAACCCCCCACCGAGCTGGAGCCTGAGCCGGAGATAAGGGGCTGCTGACA
 CCAGTGCTGGAGGCTGGCATGTGGATGAAGGACCCCTGAGCAAGGCCTCTCAGCCCCACGAGGCTG
 CAGCCAGCACTGCCACCGGAGGCACAGTCGGTCCCCAGCTGGAGGAGGTGGCACGGGTGTTGGCG
 GAAATTCCCCGGCCCCCTCAAACGCAGGGCTCCATGGAGCAGGCCCTGCTGTGGCCCTGCCCTA
 CCCACAAGAAACAGTACCAAGCAGATCATGCCGCCTTCCATCGTATGGGGGCCAGGGCCCG
 GGGCGGAGCCAGAGCTGCCCCATCACTGAGGGATCTGAGGCCAGGGCAGGGCCCCCTGCTCTG
 CCCCCAC CAGCTCCCATCCACCGCCGGCCCCGTCCCAGAGCAGCCACCAGAGCAGCCGAGAC
 ATGGAGATGCGCTCTGTGTCGGAAAGGCGGCTCCCCCGCAAGGCCGCCGCGCGCCTCAACC
 CTCTGGTGCTCCCTGGACCGCGCTGACGGGGAGCTGGAGGTGGTCAGCAGGGCGTGAAGG
 AGATGAACGACCCGAGCCAGCCAACAGAGGGCATCACTGCCCTGCACAACGCCATGCGCG
 CCAACTACTCTATCGTGGATTCTCATCACCGCGGGTCCAATGTCACCTCCCCGACAGCCACGGC
 TGGACACCTTGCACTGCGCGCTGTGCAACGACACAGTCATCTGCATGGCGCTGGTCAGCACG
 GCGCTG CAATCTTCGC CACCAACGCTC AGCGACGGCG CCACCGCCTTCGAGAAGTGCAGCCCTTAC
 GCGAGGGTTATGCTGACTGCGCCACTACCTGGCAGACGCTGAGCAGAGTATGGGCTGATGAACA
 GCGGGGAGTGTACGCTCTGGGACTACAGCGCCGAGTTGGGACAGCTGTCCCTCGCGAGGG
 CGAGTCGGTCAACCGTGTGCGGAGGGACGGCCGGAGGAGACCGACTGGTGGTGGGCCGCGTGC
 CGGCCAGGAGGGCTACGTGCCCGCGGAACTACTTGGGCTGTTCCCCAGGGTGAAGCCTCAAAGGAGT
 AAAGTCTAGCAGGATAGAAGGAGGTTCTGAGGCAGACAGAAACAGCATTCTGCCCTCCCTCCAG
 ACCTCTC CCTCTGTTTGTGCTGCCCT TATCTGCACC CCTCACCTG CTGGTGGTGG TCCTTGCCAC
 CGGTTCTCTGTTCTCTGGAAAGTCCAGGGAAAGGAGGGCCCCAGCCTAAATTAGTAATCTGCC
 TTAGCCTTGGGAGGGCTGGGAAGGGCTGGAAATCACTGGGACAGGAAACCACTTCCCTTTGCCAAA
 TCAGAT CCCGTCAAA GTGCCCTCCCA TGCCCTACAC CATCATACA TCCCCCAGCAAGGCCAGCCAC
 CTGCCAGCCGGGCTGGGATGGGCCACACACCACTGGATATTCTGGAGTCAGCTGCTGACACCA
 TCTCTCCAGCAGTCTGGGCTGGGAAACATTGGTCTACCAAGGATCCCTGCCCAACCTCT
 CCCCCA ATTAAGTGCCTTCACACAGC ACTGGTTAATGTTATAAA CAAAATAGAG AACTGGTTT
 AATGTTATA AAAAAAATAG AGAAAACCTTCTGCTTATAAA AAAAGTAGTT TGACACAGAAA
 TGAAAAAAAAA AAAAAA AAAAAA

Figure 3.1

IASPP
RAI
RAI 2.6 kb corr. translation

10 20 30 40 50 60 70

80 90 100 110 120 130 140

150 160 170 180 190 200 210

220 230 240 250 260 270 280

280 300 310 320 330 340 350

360 370 380 390 400 410 420

430 440 450 460 470 480 490

500 510 520 530 540 550 560

Peptide antigen (pAbiASPP18)

570 580 590 600 610 620 630

640 650 660 670 680 690 700

710 720 730 740 750 760 770

780 790 800 810 820 830 840

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Figure 3.2

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Figure 3.3

Sequence alignment of IASPP cDNA, RAI 2.1kb cDNA, and RAI 2.6kb cDNA from position 990 to 1880.

The alignment shows the following sequence blocks:

- Block 1 (990-1480):** IASPP cDNA, RAI 2.1kb cDNA, RAI 2.6kb cDNA
- Block 2 (1480-1510):** IASPP cDNA, RAI 2.1kb cDNA, RAI 2.6kb cDNA
- Block 3 (1510-1530):** IASPP cDNA, RAI 2.1kb cDNA, RAI 2.6kb cDNA
- Block 4 (1530-1620):** IASPP cDNA, RAI 2.1kb cDNA, RAI 2.6kb cDNA
- Block 5 (1620-1690):** IASPP cDNA, RAI 2.1kb cDNA, RAI 2.6kb cDNA
- Block 6 (1690-1760):** IASPP cDNA, RAI 2.1kb cDNA, RAI 2.6kb cDNA
- Block 7 (1760-1880):** IASPP cDNA, RAI 2.1kb cDNA, RAI 2.6kb cDNA

Annotations in the alignment:

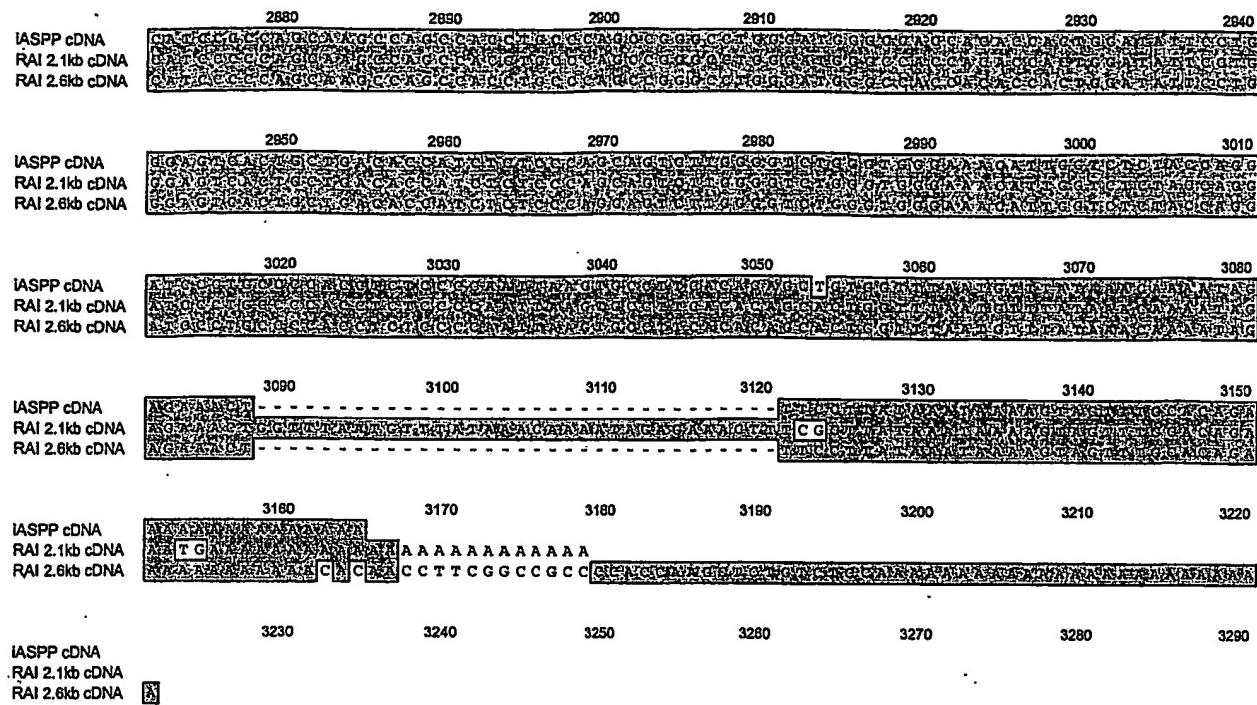
- RAI:** An arrow points to the RAI gene name located above the sequence at position 1510.
- Stop codon introduced by frameshift:** An arrow points to the sequence at position 1510, indicating a frameshift that creates a stop codon.
- Frameshift – see protein sequence:** An arrow points to the sequence at position 1760, indicating a frameshift that changes the reading frame.

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Figure 3.4

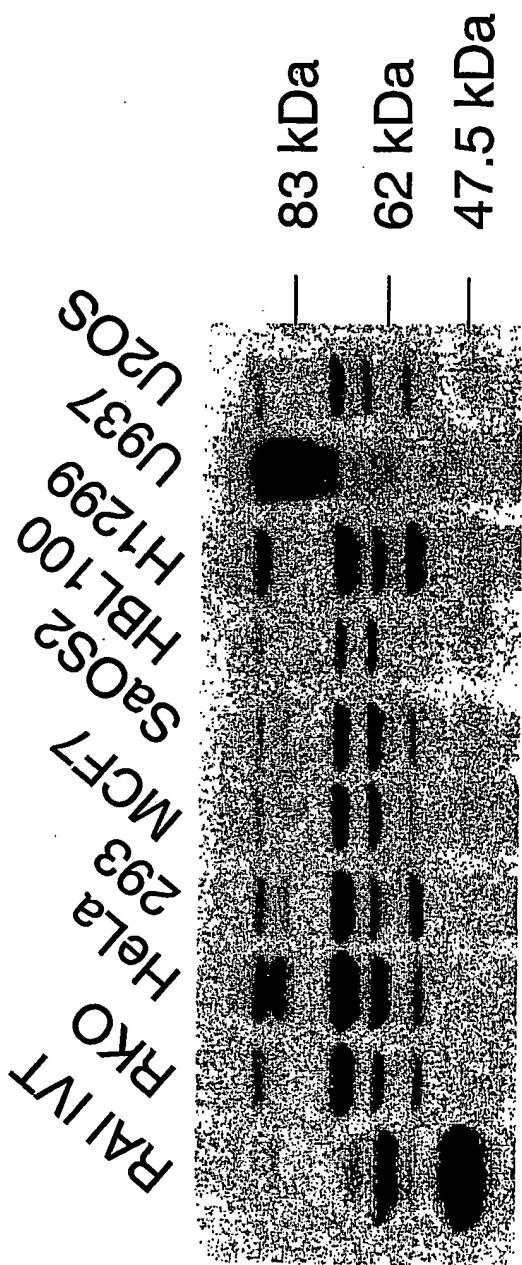
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Figure 3.5



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Expression of iASPP in various cell lines



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Figure 4B

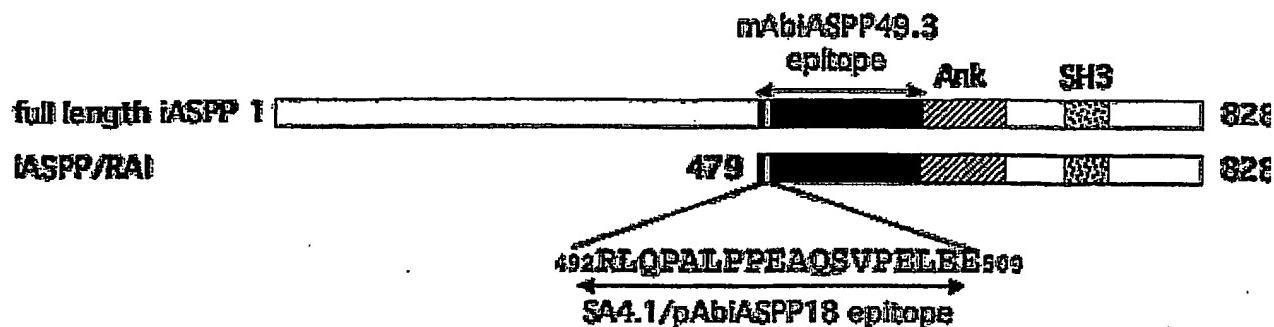


Figure 4C

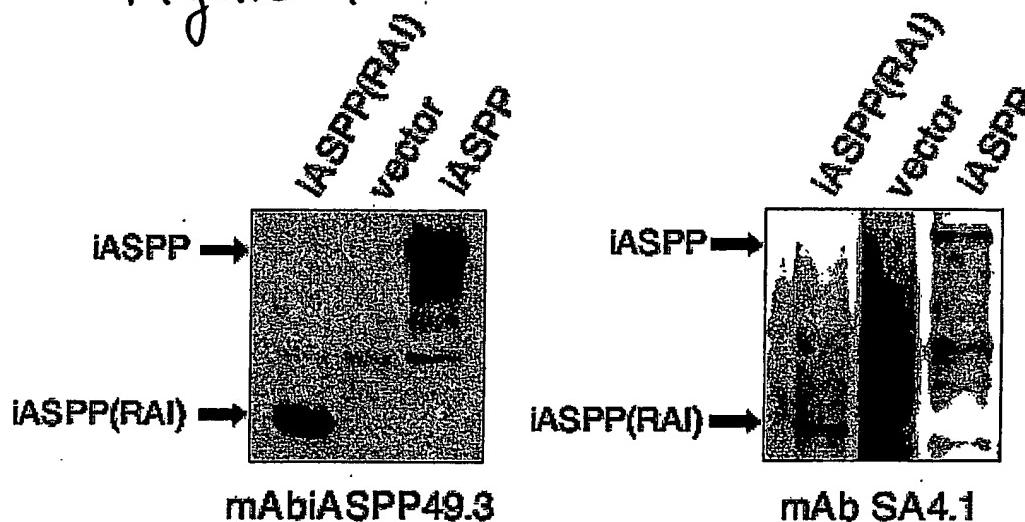
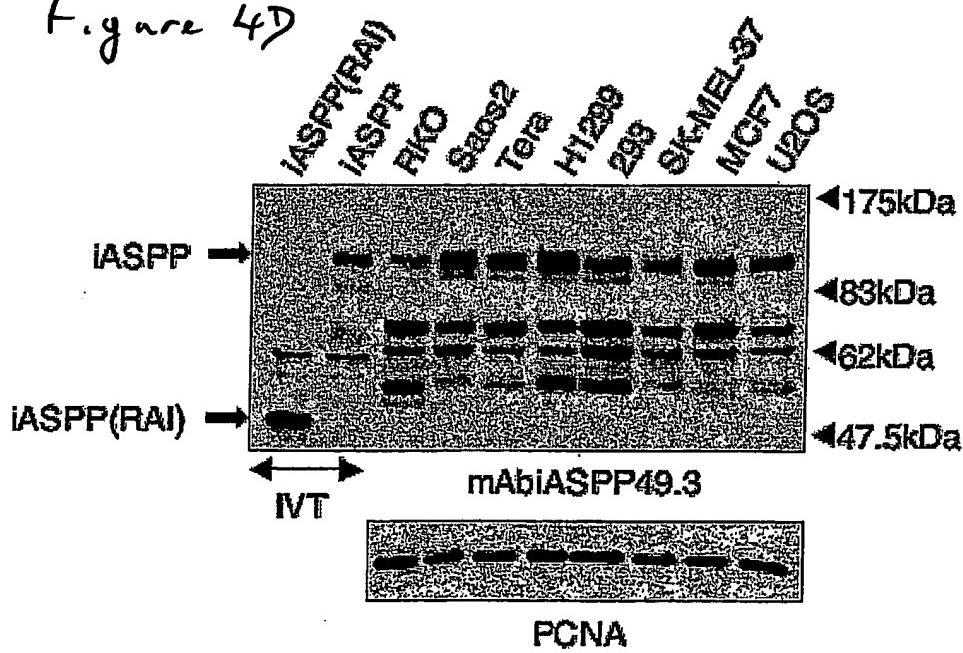
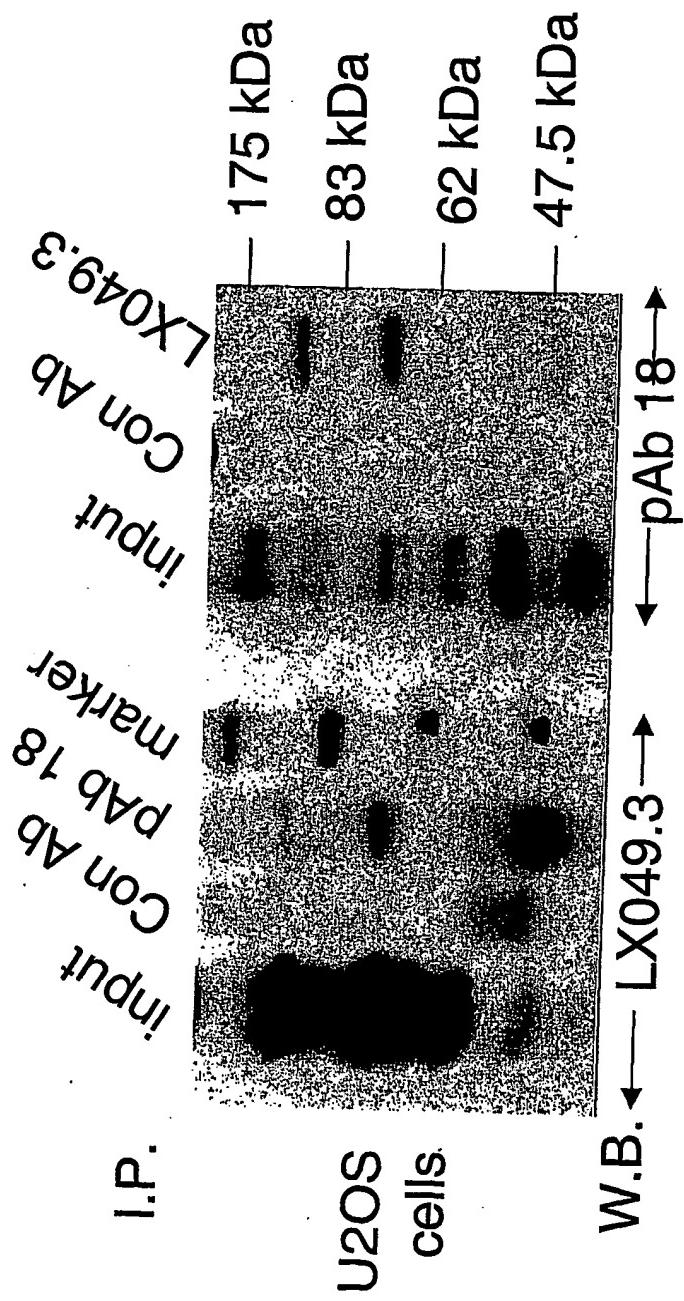


Figure 4D



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iASPP - I.P./Western blot



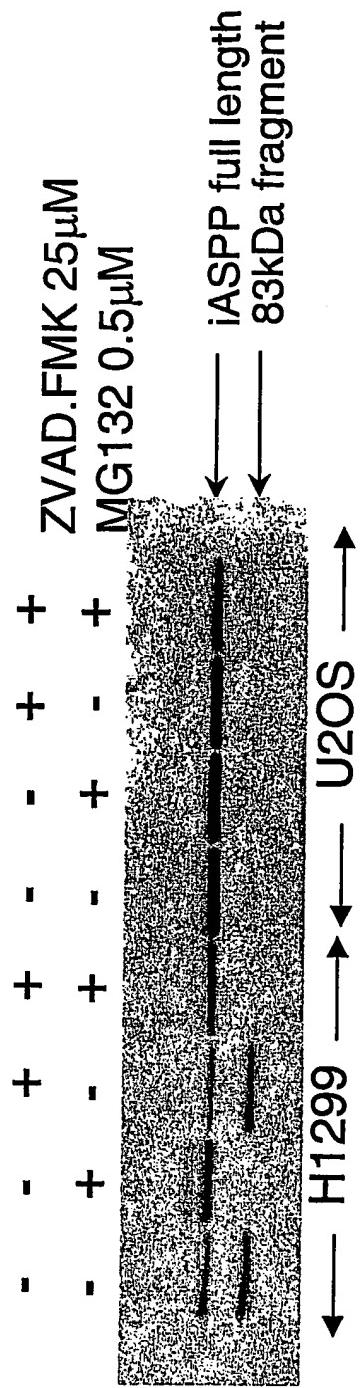
Effect of cell density and MG132 upon iASPP expression in U2OS cells

Figure b a



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Effect of MG132, Z-VAD.FMK upon iASPP



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Interaction of iASPP with p53 and Bcl2 in U2OS cells

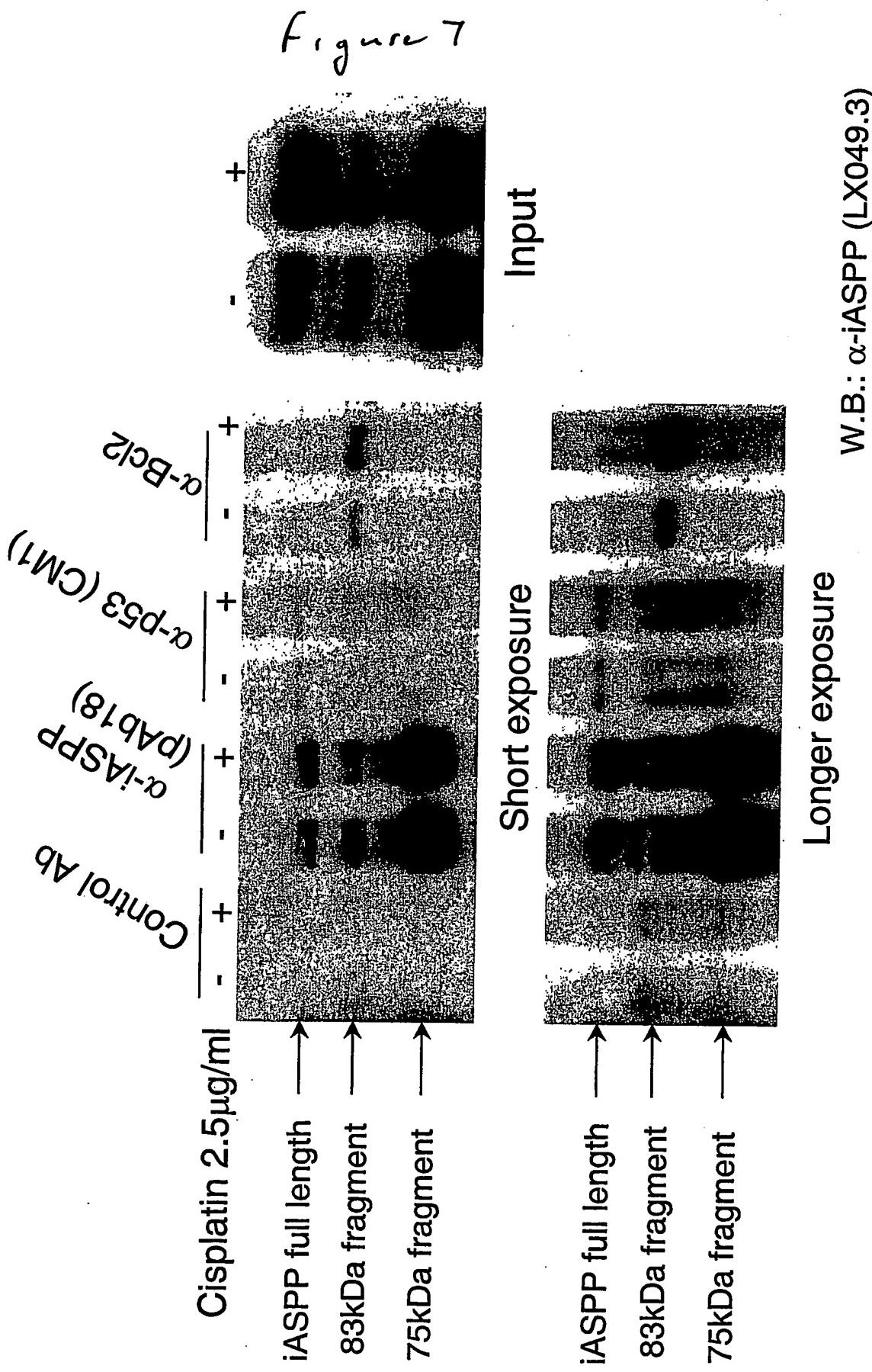
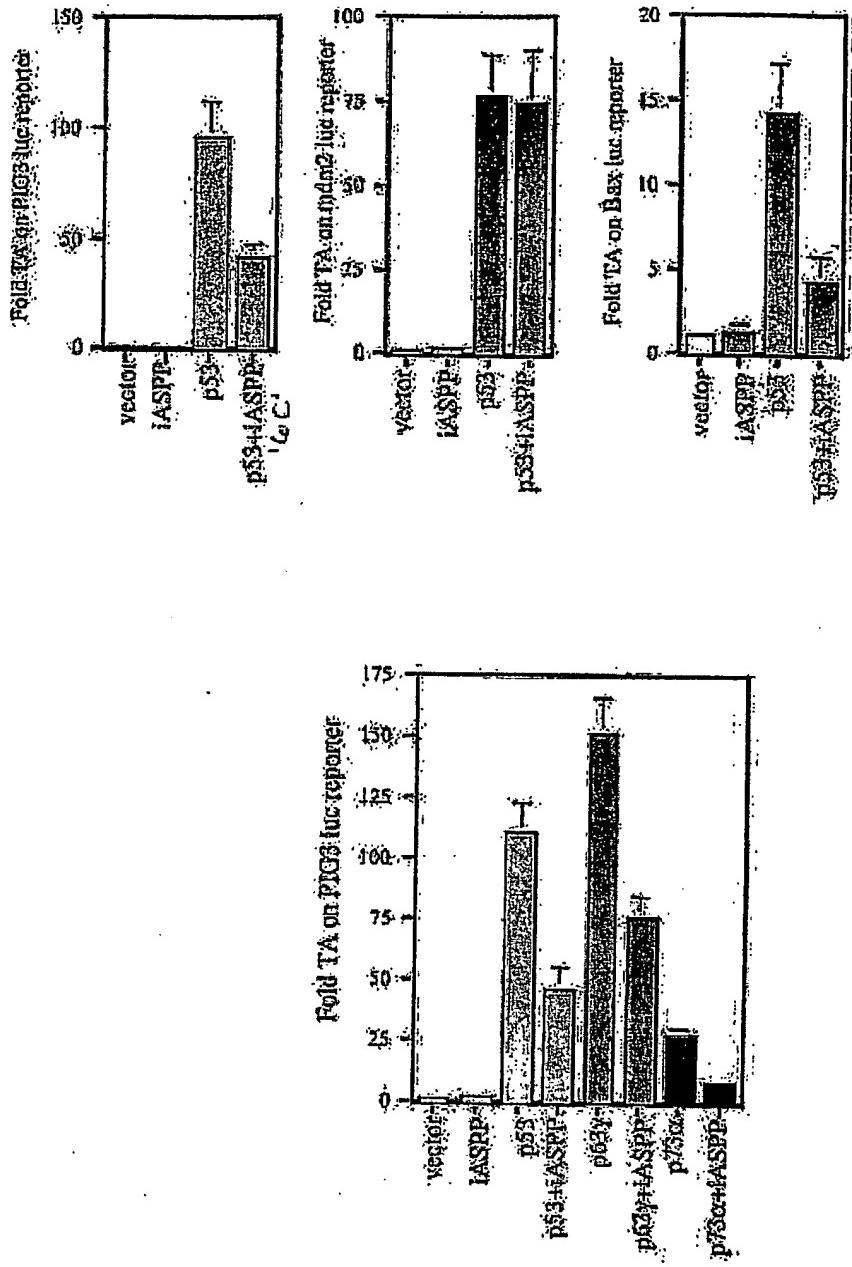


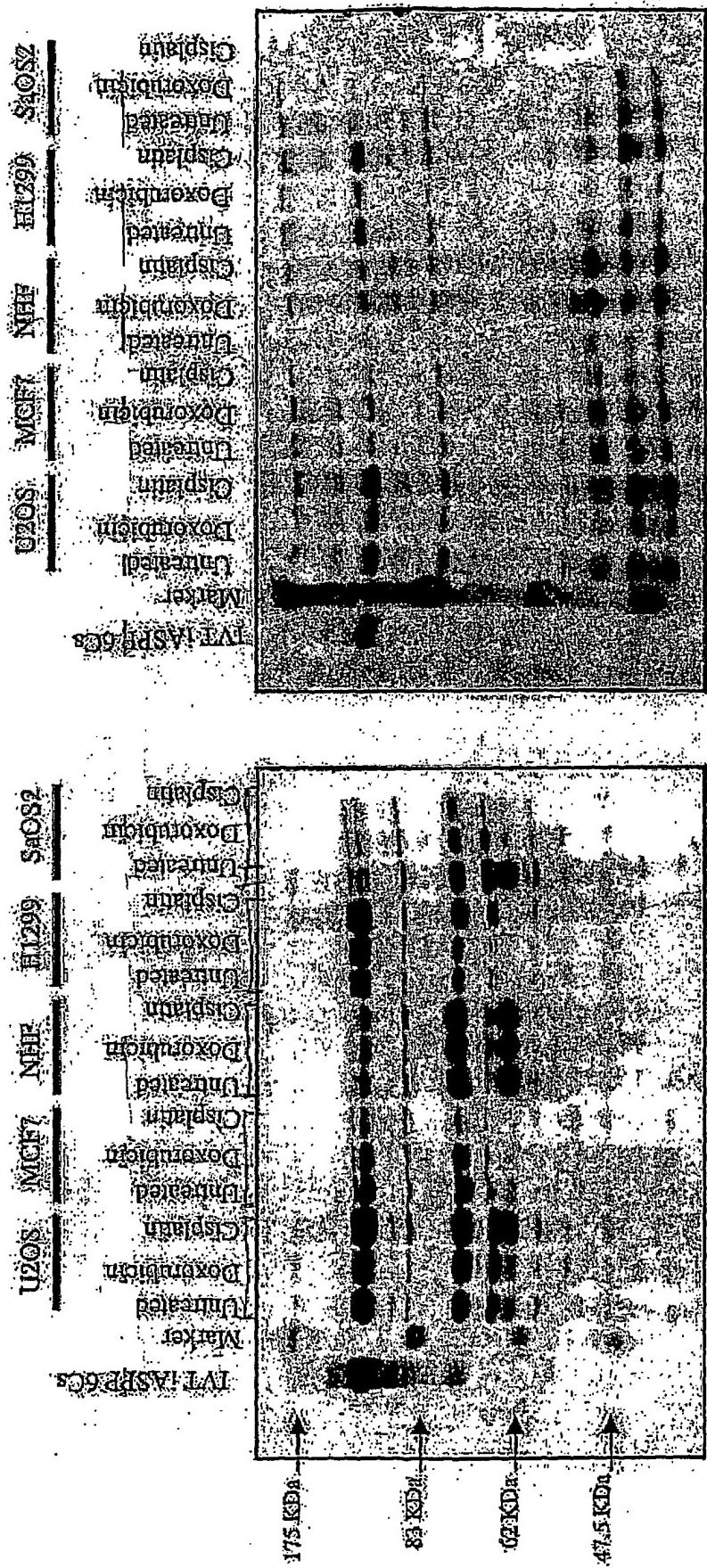
Figure 8



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Figure 9

LASEP pattern in five different cell lines



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